

### Claims

1. A peptide which binds streptavidin with a dissociation constant less than 10  $\mu\text{M}$ , wherein said peptide is not disulfide bonded or cyclized.

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2. A peptide which binds streptavidin with a dissociation constant less than 10  $\mu\text{M}$ , wherein the amino acid sequence of said peptide does not contain an HPQ, HPM, HPN, or HQP motif.

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3. A peptide which binds streptavidin with a dissociation constant less than 23 nM.

4. The peptide of claim 1 or 2, wherein said dissociation constant is less than 5  $\mu\text{M}$ .

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5. The peptide of claim 1 or 2, wherein said dissociation constant is less than 1  $\mu\text{M}$ .

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6. The peptide of claim 5, wherein said dissociation constant is less than 100 nM.

7. The peptide of claim 6, wherein said dissociation constant is less than 50 nM.

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8. The peptide of claim 3, wherein said dissociation constant is less than 10 nM.

9. The peptide of claim 8, wherein said dissociation constant is less than 5 nM.

10. The peptide of any one of claims 1-3, comprising at least 10 consecutive amino acids of any one of SEQ ID Nos. 1-29.

11. The peptide of claim 10, comprising at least 25 consecutive amino acids of any one of SEQ ID Nos. 1-29.

12. The peptide of claim 11, comprising at least 50 consecutive amino acids of any one of SEQ ID Nos. 1-29.

13. The peptide of claim 12, comprising at least 100 consecutive amino acids of any one of SEQ ID Nos. 1-29.

14. The peptide of any one of claims 1-3, comprising the amino acid sequence of any one of SEQ ID Nos. 1-29 or 35.

15. A nucleic acid encoding a peptide of any one of claims 1-3.

16. A vector comprising a nucleic acid of claim 15.

17. A fusion protein comprising a protein of interest covalently linked to:  
(a) a peptide which binds streptavidin with a dissociation constant less than 10  $\mu$ M, wherein said peptide is not disulfide bonded or cyclized;

(b) a peptide which binds streptavidin with a dissociation constant less than 10  $\mu$ M, wherein said peptide does not contain an HPQ, HPM, HPN, or HQP motif; or

(c) a peptide which binds streptavidin with a dissociation constant less than 23 nM.

18. The fusion protein of claim 17, wherein said peptide is attached to the amino terminus or the carboxy terminus of said protein of interest, or wherein said

peptide is positioned between the amino and carboxy termini of said protein of interest.

19. The fusion protein of claim 17, wherein said peptide is linked to said protein of interest by a linker comprising a protease-sensitive site.

20. A nucleic acid encoding a fusion protein of claim 17.

21. A vector comprising a nucleic acid of claim 20.

22. A method of producing a streptavidin-binding fusion protein, said method comprising the steps of:

(a) expressing in a host cell, a gene encoding a fusion protein of claim 17 and

(b) culturing said host cell under conditions appropriate for production of said fusion protein.

23. A method of purifying a protein of interest from a sample, said method comprising the steps of:

(a) expressing in said sample, a fusion protein comprising said protein of interest covalently linked to:

(i) a peptide which binds streptavidin with a dissociation constant less than 10  $\mu$ M, wherein said peptide is not disulfide bonded or cyclized;

(ii) a peptide which binds streptavidin with a dissociation constant less than 10  $\mu$ M, wherein said peptide does not contain an HPQ, HPM, HPN, or HQP motif; or

(iii) a peptide which binds streptavidin with a dissociation constant less than 23 nM;

(b) contacting said sample with streptavidin under conditions that allow complex formation between said fusion protein and said streptavidin;

(c) isolating said complex; and  
(d) recovering said fusion protein, thereby purifying said protein of interest from said sample.

5           24. A method of detecting the presence of a fusion protein of claim 17 in a sample, said method comprising the steps of:

(a) contacting said sample with streptavidin, or streptavidin-containing compounds, under conditions that allow complex formation between said fusion protein and said streptavidin, or streptavidin-containing compounds;

10           (b) isolating said complex; and

(c) detecting the presence of said streptavidin, or streptavidin-containing compounds, wherein the presence of said streptavidin, or streptavidin-containing compounds, indicates the presence of said fusion protein in said sample.

15           25. The method of claim 24 wherein step (c) comprises detecting the presence of said streptavidin in said complex

20           26. The method of claim 24 wherein step (c) comprises detecting the presence of said streptavidin recovered from said complex.

25           27. The method of claim 24 wherein step (c) further comprises measuring the amount of said streptavidin, wherein the amount of said streptavidin is correlated with the amount of said fusion protein in said sample.